PCT/US01/11487

## What is claimed is:

WO 01/77178

- 1. An isolated nucleic acid molecule comprising a nucleotide sequence encoding mutated canine von Willebrand's Factor polypeptide which causes canine von Willebrand's disease, wherein the nucleotide sequence is capable of hybridizing under high stringency conditions to the complementary sequence of the sequence of SEQ ID NO. 1 having a mutation at nucleotide 172, or SEQ ID NO. 2 having a mutation at nucleotide 384.
- 2. A vector comprising the nucleic acid molecule of claim 1.
- 3. A cell comprising the vector of claim 2.
- 4. The isolated nucleic acid molecule of claim 1, wherein the mutation at nucleotide 172 of SEQ ID NO:1 is a nucleic acid substitution.
- 5. The isolated nucleic acid molecule of claim 1, wherein the mutation at nucleotide 384 of SEQ ID NO:2 is a nucleic acid deletion.
- 6. A method of detecting a canine von Willebrand's Factor gene in a sample comprising the steps of:
  - a) contacting the sample with an oligonucleotide comprising at least 10 contiguous nucleotides derived from the nucleic acid sequence of SEQ ID NO:1 or complement thereof, and capable of specifically hybridizing with the canine von Willebrand's Factor gene, under conditions favorable for hybridization of the oligonucleotide to any complementary sequence of nucleic acid in the sample; and
  - b) detecting hybridization, thereby detecting a canine von Willebrand's Factor gene.
- 7. The method of claim 6, further comprising the step of:
  - c) quantifying hybridization of the oligonucleotide to the complementary sequence.

8. The method of claim 6, wherein SEQ ID NO:1 has a nucleic acid substitution at nucleotide 172.

- 9. A method of detecting a canine von Willebrand's Factor gene in a sample comprising the steps of:
  - a) contacting the sample with an oligonucleotide comprising at least 10 contiguous nucleotides derived from the nucleic acid sequence of SEQ ID NO:2 or complement thereof, and capable of specifically hybridizing with the canine von Willebrand's Factor gene, under conditions favorable for hybridization of the oligonucleotide to any complementary sequence of nucleic acid in the sample; and
  - b) detecting hybridization, thereby detecting a canine von Willebrand's Factor gene.
- 10. The method of claim 9, further comprising the step of:
  - c) quantifying hybridization of the oligonucleotide to the complementary sequence.
- 11. The method of claim 9, wherein SEQ ID NO:2 has a nucleic acid deletion at nucleotide 384.
- 12. An assay kit for screening for a canine von Willebrand's Factor gene comprising:
  - a) an oligonucleotide comprising at least 10 contiguous nucleotides of the nucleic acid sequence of SEQ ID NO:1, and capable of hybridizing with the nucleotide sequence encoding canine von Willebrand's Factor;
  - b) reagents for hybridization of the oligonucleotide to a complementary nucleic acid sequence; and
  - c) container means for a) and b).
- 13. The assay kit of claim 12, wherein SEQ ID NO:1 has a nucleic acid substitution at nucleotide 172.
- 14. An assay kit for screening for a canine von Willebrand's Factor gene comprising:

a) an oligonucleotide comprising at least 10 contiguous nucleotides of the nucleic acid sequence of SEQ ID NO:2, and capable of hybridizing with the nucleotide sequence encoding canine von Willebrand's Factor;

- b) reagents for hybridization of the oligonucleotide to a complementary nucleic acid sequence; and
- c) container means for a) and b).
- 15. The assay kit of claim 14, wherein SEQ ID NO:2 has a nucleic acid deletion at nucleotide 384.
- 16. An assay kit for screening for a canine von Willebrand's Factor gene comprising:
  a) an oligonucleotide comprising contiguous nucleotides of the nucleic acid sequence that is complementary to the sequence of SEQ ID NO:1 having a mutation at nucleotide 172, and capable of specifically hybridizing to the complementary nucleotide sequence;
  - b) reagents for hybridization of the oligonucleotide to a complementary nucleic acid sequence; and
  - c) container means for a) and b).
- 17. The assay kit of claim 16, wherein the mutation at nucleotide 172 is a nucleic acid substitution.
- 18. An assay kit for screening for a canine von Willebrand's Factor gene comprising:

  a) an oligonucleotide comprising contiguous nucleotides of the nucleic acid sequence that is complementary to the sequence of SEQ ID NO:2 having a mutation at nucleotide 384, and capable of specifically hybridizing to the complementary nucleotide sequence;

b) reagents for hybridization of the oligonucleotide to a complementary nucleic acid sequence; and

- c) container means for a) and b).
- 19. The assay kit of claim 18, wherein the mutation at nucleotide 384 is a nucleic acid deletion.
- 20. A method for detecting a mutated canine von Willebrand's Factor gene in a canine DNA sample comprising the steps of:
  - a) amplifying the DNA sample by polymerase chain reaction to produce polymerase chain reaction products, wherein the polymerase chain reaction uses primers that produce a restriction site in a normal allele but not in a mutant allele, wherein the mutation in the mutant allele is a substitution at nucleotide 172 of the nucleotide sequence encoding canine von Willebrand's Factor polypeptide, wherein the nucleotide sequence is capable of hybridizing under high stringency conditions to the complementary sequence of the sequence of SEQ ID NO:1;
  - b) digesting the polymerase chain reaction products with a restriction enzyme specific to the restriction site of the primers to produce DNA fragments; and
  - c) detecting the DNA fragments, thereby detecting a mutated canine von Willebrand's Factor gene.
- 21. The method of claim 20, wherein the DNA fragments are detected by gel electrophoresis.
- 22. The method of claim 20, wherein the primers comprise the sequence of SEQ ID NO:3 and SEQ ID NO:4.
- 23. The method of claim 22, wherein the restriction enzyme is Tag I.
- 24. The method of claim 20, wherein the primers comprise the sequence of SEQ ID NO:3 and SEQ ID NO:9.

- 25. The method of claim 24, wherein the restriction enzyme is Hph I.
- 26. A method for detecting a mutated canine von Willebrand's Factor gene in a canine DNA sample comprising the steps of:
  - a) amplifying the DNA sample by polymerase chain reaction to produce polymerase chain reaction products, wherein the polymerase chain reaction uses primers that are complementary to sequences of the introns flanking the exon, wherein the exon of the mutant allele has a deletion at nucleotide 384 of the nucleotide sequence encoding canine von Willebrand's Factor polypeptide, wherein the nucleotide sequence is capable of hybridizing under high stringency conditions to the complementary sequence of the sequence of SEQ ID NO:2;
  - b) digesting the polymerase chain reaction products with a restriction enzyme specific to the restriction site of the mutant allele to produce DNA fragments; and
  - c) detecting the DNA fragments, thereby detecting a mutated canine von Willebrand's Factor gene.
- 27. The method of claim 26, wherein the DNA fragments are detected by gel electrophoresis.
- 28. The method of claim 26, wherein the primers comprise the sequence of SEQ ID NO:5 and SEQ ID NO:6.
- 29. The method of claim 26, wherein the primers comprise the sequence of SEQ ID NO:7 and SEQ ID NO:8.
- 30. The method of claim 28, wherein the restriction enzyme is Mwo I.
- 31. The method of claim 29, wherein the restriction enzyme is Mwo I.

32. An oligonucleotide probe capable of detecting a mutation associated with canine von Willebrand's disease, wherein the mutation is a base substitution at nucleotide 172 of the nucleotide sequence encoding canine von Willebrand's Factor polypeptide, wherein the nucleotide sequence is capable of hybridizing under high stringency conditions to the complementary sequence of the sequence of SEQ ID NO. 1.

33. The oligonucleotide probe of claim 32, wherein the substitution at nucleotide 172 is adenine for guanine.